



# Effect of Synbiotic Supplementation on Maternal and Neonatal Outcomes in Pregnant Women With Pre-eclampsia: Study Protocol for a Triple Blind Randomized Controlled Clinical Trial

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## Abstract

**Objectives:** Background: Preeclampsia is one of the main causes of premature birth, growth restriction, and intrauterine death of fetus. Probiotics has the potential to modulate inflammatory and oxidative stress biomarkers that implicated in the pathophysiology of preeclampsia. The aim of the present study is to establish the impact of synbiotic supplements, comprising of probiotic and prebiotic fructooligosaccharide, in comparison to placebo, on the maternal and neonatal outcome outcomes in women afflicted with mild preeclampsia.

**Methods:** This is a study protocol of a randomized, controlled, phase 3, triple-blind, randomized clinical trial. The classification is based on the gestational age at the time of diagnosis of mild preeclampsia (early-onset or late-onset preeclampsia). Participants will be 128 pregnant women with mild pre-eclampsia (systolic blood pressure between 140-160 mm Hg or diastolic blood pressure between 90-110 mm Hg, along with other preeclampsia symptoms). Participants will divide into two intervention and control groups using a 1:1 random allocation ratio randomly. They will receive one oral capsule (the concentration of  $10^9$  CFU) or placebo daily from admission until delivery. Primary outcomes included mean systolic and diastolic blood pressure, mean gestational age from diagnosis to delivery, and mean birth weight. Also, secondary outcomes included proteinuria, serum creatinine level, the incidence of severe PE, the use of antihypertensive drugs, the rate of natural delivery, incidence of serious complications, maternal blood factors such as platelet count, and serum levels of liver enzymes such as ALT, AST, bilirubin, and LDH.

**Discussion:** The present trial can importantly contribute to the selection of an appropriate Synbiotic supplement as safe pharmaceutical adjuvants in the treatment of pregnant women with mild preeclampsia and prevention of maternal and neonatal complications.

**Trial Registration:** IRCT20110606006709N20. Registered on August 13, 2022.

**Keywords:** Preeclampsia, Gestational hypertension, Probiotic, Synbiotic, Pregnancy outcomes

## Introduction

Pregnancy represents a crucial stage in which complications may arise, including hypertensive disorders and notably preeclampsia. It is important to note that this disorder is among the top three causes of maternal and fetal mortality worldwide, along with bleeding and infection (1).

The overall prevalence rate of preeclampsia and eclampsia is 6.7%, and highest prevalences is in low-and medium-income countries (2). Preeclampsia is the leading cause of high-risk pregnancy, postpartum admission to the prenatal care unit (3).

Although some supplements such as aspirin, magnesium, and vitamins E and C have been suggested to prevent in high-risk mothers (4-6), since the cause and pathogenesis of preeclampsia are not fully understood, no prevention or immediate treatment for preeclampsia

has been formally proposed (7). The prevention and treatment of preeclampsia pose significant challenges, as no dependable and expeditious measures currently exist. The field of therapeutics remains fraught with difficulties, requiring further study and exploration (8). Therefore, for early-onset preeclampsia, physicians often have to terminate the pregnancy to prevent maternal morbidity, but severe neonatal morbidity will increase (9).

Multiple evidence suggests that preeclampsia and gestational hypertension have various demonstrations, including vascular changes, vasospasm, and disorders in body systems due to maternal vascular endothelium pathology (10). Among the pathogens proposed for preeclampsia, endothelial disorder and inflammatory response are an important part of the pathogenesis of preeclampsia, which can also be caused by oxidative

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## Key Messages

- The present trial can contribute to selecting an appropriate Synbiotic supplement composed of seven probiotic strains of bacteria consisting *Lactobacillus casei*, *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Lactobacillus bulgaricus*, *Bifidobacterium breve*, *Bifidobacterium longum*, and *Streptococcus thermophilus* alongside Fructooligosaccharides prebiotics as a safe pharmaceutical adjuvant in the treatment of pregnant women with mild preeclampsia and the prevention of its subsequent maternal and neonatal complications. It is suggested to consider a probiotic group in addition to the synbiotic and placebo groups in future studies.

stress (11,12). Evidence shows that maternal excessive inflammatory response is responsible for preeclampsia (13). Elevated levels of oxidative stress during pregnancy have been found to be linked with multiple adverse consequences, including preeclampsia (14), low birth weight (LBW), preterm labor and thrombocytopenia (15). Also, placental dysfunction leads to a localized and systemic increment in oxidative stress in the mother, which leads to symptomatic hypertension with maternal vascular endothelial dysfunction (16).

Imbalance in the gut microflora can upset the ratio of gram-positive and gram-negative bacteria and eventually causing inflammation (17).

Probiotics are known as living microorganisms that, if taken enough, will have beneficial effects on the host (18). These supplements play a positive role in reducing systemic inflammation and oxidative stress (19) and they are known to be beneficial to gastrointestinal health by suppressing pathogenic bacteria as well as influencing physiological processes involved in blood pressure, inflammation, kidney function, and diabetes (20). Probiotics have been proposed to modulate placental trophoblast inflammation, systemic inflammation, and hypertension, which are significant aspects of preeclampsia (21). By mechanisms such as lowering total cholesterol, low density lipoprotein, triglycerides, blood glucose, insulin resistance, and stabilising the renin-angiotensin system, these supplements may improve blood pressure (20).

Probiotics are naturally found in the human gut and do not seem to pose any safety concerns for pregnant and lactating women. When healthy people use probiotics, systemic absorption is rare and studies do not show an increase in adverse outcomes during pregnancy and lactation (22).

Prebiotics, which selectively promote the growth and activity of beneficial bacteria in the gut, are suggested as nutrient fibers of probiotics and components of indigestible carbohydrates (23).

The aim of this study is to determine the effect of synbiotic supplements (probiotic with prebiotic) in comparison with placebo on maternal, neonatal outcomes in mothers with

mild preeclampsia. Given most studies in this field have been conducted in an observational retrospective design, we will try to evaluate the effect of these supplements on the course of gestational hypertension and preeclampsia and other pregnancy outcomes with great accuracy.

### Materials and Methods

This is a study protocol of a randomized, controlled, phase III, triple-blind, randomize clinical trial. Stratification shall be executed with respect to gestational age during the point of diagnosis of mild preeclampsia, distinguished as early-onset or late-onset preeclampsia. Envelopes numbered 1 to 100 will be allocated to late-onset preeclampsia, and envelopes 100 to 128 will be allocated to early-onset preeclampsia. Participants, researchers, outcome evaluators, and statistical analysts will not be aware of the type of intervention received. The research population will be pregnant women referring to the high risk pregnancy clinic of Tabriz University of Medical Sciences. The research samples comprise 128 expectant mothers in their second trimester who have been diagnosed with mild preeclampsia and will be directed to the high-risk pregnancy clinic at this medical facility.

### Inclusion Criteria

- 1) The diagnosis of mild preeclampsia is established when systolic blood pressure reaches or exceeds 140 mm Hg or diastolic blood pressure reaches or exceeds 90 mm Hg for the first time after the 20th week of pregnancy, which is accompanied by proteinuria or multi-organ involvement, such as renal, liver, nervous system dysfunction or pulmonary edema (13).
- 2) Single pregnancy with a live fetus
- 3) Gestational age over 24 weeks
- 5) Maternal age less than 45 years
- 6) The suitability of maternal and fetal conditions for expectant management.

### Exclusion Criteria

- 1) Women who have been diagnosed with cardiovascular diseases
- 2) Women with hepatic and renal failure
- 3) Severe and chronic high blood pressure
- 4) Gestational and chronic diabetes
- 5) A history of allergy to probiotics or synbiotics
- 6) Acute gastrointestinal problems
- 7) Use of antibiotics during the study or the past two weeks
- 8) Use of glucocorticoid and immunosuppressive drugs (unless corticosteroids are prescribed for fetal lung maturation).
- 9) Maternal or fetal conditions that require immediate delivery. Conditions unrelated to preeclampsia (such as premature rupture of the sac or true umbilical cord knot leading to severe fetal distress) and conditions related to preeclampsia (such as fetal intrauterine growth restriction, percentile growth of less than 10%, color

Doppler ultrasonography disorder, placenta abruption, pulmonary edema, and eclampsia).

10) Intake of probiotic or synbiotic supplements and foods such as probiotic yogurt in the last three months.

### Intervention type

The intervention group will receive LactoCare synbiotic capsule manufactured by ZistTakhmir Company. The probiotic and prebiotic strains will be included:

- *Lactobacillus casei*:  $3 \times 10^9$
- *Lactobacillus acidophilus*:  $3 \times 10^9$
- *Lactobacillus rhamnosus*:  $7 \times 10^9$
- *Lactobacillus bulgaricus*:  $5 \times 10^8$
- *Bifidobacterium longum*:  $1 \times 10^9$
- *Bifidobacterium breve*:  $2 \times 10^{10}$
- *Streptococcus thermophilus*:  $3 \times 10^8$
- Fructooligosaccharides: 38.5 mg

The control group will receive the placebo inactive capsules, which will be similar in appearance and color to the synbiotic capsules made by the ZistTakhmir Company and include maltodextrin, lactose magnesium stearate. Both groups will take the capsules from the time of enrollment until delivery. All trial participants will receive usual care usual care in addition to the study capsules. Both groups will be advised not to take probiotic products during the intervention other than the capsules received in this study and reduce salt intake and physical activity.

### Sample Size

The calculation of the sample size was based on the consideration all four variables of birth weight, blood pressure (systolic and diastolic), and duration of pregnancy from diagnosis to delivery by G\*Power software (version 3.1.2) using the difference between two independent means formula. Based on the study by Chappell et al (24) for birth weight variable (gram), taking into account mean<sub>1</sub>= 2670, mean<sub>2</sub>=2803, standard deviation (sd)<sub>1</sub>=sd<sub>2</sub>=234.25, power 80%,  $\alpha=0.05$  and two-tailed test, the sample size calculated 55 individuals for each group considering a 10% drop out.

Concerning diastolic blood pressure variables (mm Hg): mean<sub>1</sub>=107, mean<sub>2</sub>= 96.3, sd<sub>1</sub>=sd<sub>2</sub>=12 power 80%,  $\alpha=0.05$  and two-tailed test, 23 participants was calculated assuming 10% sample drop for each group.

According to the study by Chappell et al (24) on the variable of gestational age at delivery (day) and taking into account mean<sub>1</sub>=262/5 and mean<sub>2</sub>=267.5, sd<sub>1</sub>=sd<sub>2</sub>=7.35, power 80%,  $\alpha=0.05$  and two-tailed tests, the sample size was calculated 39 people for each group considering the 10% drop out.

According to the study by Cluver et al (25) on the duration of pregnancy from diagnosis to delivery (day) and taking into account power 80%, with mean<sub>1</sub>=8.3, mean<sub>2</sub>=10.3 and sd<sub>1</sub>=sd<sub>2</sub>=3.95,  $\alpha=0.05$ , the sample size calculated 64 individuals for each group (the obtained maximum sample size), considering a 10% sample drop.

### Data Collection Tools

1. Eligibility criteria checklist: It includes the inclusion and exclusion criteria that individuals will complete at the beginning of the study to enter the study if they qualify.
2. Demographic and obstetric questionnaire: It includes demographic and pregnancy-delivery information (questionnaire code, age, education, spouse's education, occupation, income, smoking, alcohol, and drug use, number of pregnancies, abortion, last menstrual period (LMP), weight, height, etc.) that people will complete after entering the study.
3. Daily medication checklist and medication side effects: This checklist, along with training on how to use the medication or placebo, will be marked by the participants whenever the daily capsule is used in the relevant table indicating the days of the week. At the end of the questionnaire, a list of side effects has been listed which will be marked in case of complications.
4. Mothers' food recall form: This diary will be delivered to the participants at the beginning of the study to record the food consumed within three days before admission.
5. Pregnancy, delivery, and neonatal outcomes questionnaire: It includes the information on pregnancy and childbirth (gestational age at delivery based on early ultrasound, in its absence by the LMP, pretreatment test results, type of delivery, premature rupture of membranes, any serious complications such as at least one cases: Stroke, kidney failure, liver failure, HELLP syndrome, intravascular coagulation, pulmonary edema, severe preeclampsia, antihypertensive drug use), and neonatal characteristics (Apgar score less than seven min, weight, Height, fetal head circumference, fetal death, neonatal death, preterm birth, neonatal intensive care unit over seven days, LBW, the use of surfactants, intubated and mechanical ventilation).
6. Laboratory tests record sheet: Urine protein, serum creatinine, complete blood count (CBC), platelet, liver enzymes (alanine aminotransferase [ALT], aspartate aminotransferase [AST]), bilirubin, lactate dehydrogenase (LDH) will be recorded in this sheet
7. Blood pressure record sheet: blood pressure will be measured in a sitting position at hospital admission, once every 4 hours using the ISOMED mercury barometer with  $\pm 3$  mm Hg error, from the right hand. So that the arm should be placed at the heart level it will be recorded separately for systolic and diastolic blood pressure.
8. Checklist of side effects of the drug: Content validity will be used in this study to obtain the scientific validity of the tools.

### Procedure

The researcher will prepare the questionnaires according

to the purpose of the study by studying the authoritative books and journals. Ten members of the faculty of Tabriz University of Medical Sciences will be given questionnaires to review, provide comments on, and finally confirm.

The questionnaires will then be used for research after approval of their validity and reliability.

Initially, the sampling will be done in an easy or accessible manner, so that the researcher will refer to the high-risk pregnancy clinics of Al-Zahra hospital affiliated to Tabriz University of Medical Sciences. After introducing himself and outlining the purpose of the study and briefly explaining the process of conducting the research and eligibility criteria, the researcher will proceed with the initial enrollment of those interested in participating in the study. The study population will be pregnant women diagnosed with mild preeclampsia. First, all participants will be visited by a perinatologist and then the researcher will review the inclusion criteria and complete the relevant checklist for each patient.

The individuals who take part in the study shall be split into two distinct categories - synbiotic and placebo - via randomized blocks, with an allocation ratio of 1:1. The allocation sequence will be determined by a researcher not involved in this study using RAS (Random Allocation Software).

Equal to the number of samples, envelopes will be provided. Each envelope will be numbered from 1 to 128. The envelopes will be standardized, sealed, opaque, and consist of 14 capsules. The preparation of them will be carried out by an uninvolved individual in the research (Figure 1).

The Stratification will be based on gestational age during

mild preeclampsia diagnosis (early or late preeclampsia). In this study, the main and placebo supplements will be similar in shape, color, odor, and dose, and the researcher, patients, and data analyzer will be blinded. The enrollment, interventions, and evaluations schedule is presented in Figure 2.

If the 6-month period is not sufficient for sampling, the length of the sampling period will be increased. Also we will refer to Taleghani hospital in Tabriz affiliated to Tabriz University of Medical Sciences. The researcher will introduce the study objectives to all eligible women and, after obtaining written and signed informed consent from the patients, will complete the basic information form. The initial envelope will be distributed to the first qualified individual and the procedure will proceed until the sample is finished. The patients will be taught how to use the medication correctly so that from the time of enrollment until the end of pregnancy take one capsule with a glass of water every day after meals before bedtime. So that, every day after meals, before going to bed, take a capsule with a glass of water.

During the intervention period, the correct use and daily side effects will be monitored by the research team (perinatologist, head of the high-risk pregnancy department who will visit the participants daily, researcher and colleague of the high-risk pregnancy ward) who will be blinded. Individuals will receive a table with the days of the week and must mark it daily after taking capsules. After each envelope, they should return the empty one to the researcher. They will then receive the next envelope. In both groups, synbiotic and placebo capsules will be used in addition to usual care and treatment according to the

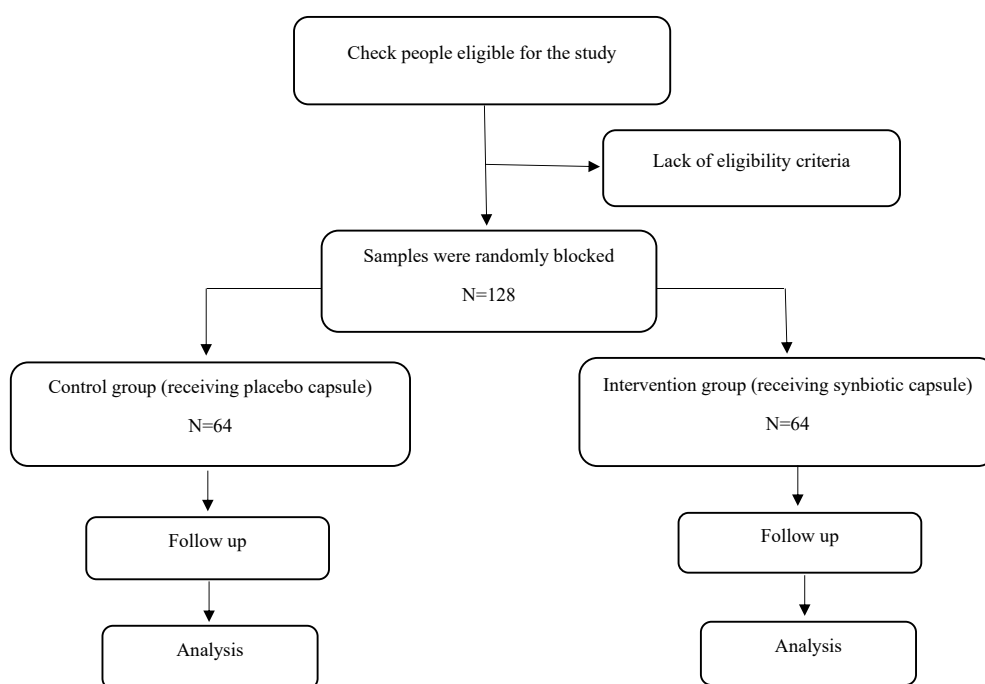


Figure 1. Enrollment Flowchart.

Timepoint	Study Period				
	Enrolment	Allocation	Post-allocation		Close-out
	Refer to the clinic	0	Start sampling	At delivery	30 days after delivery
<b>Enrolment:</b>					
Eligibility screen	X				
Informed consent	X				
Stratification		X			
Allocation		X			
<b>Interventions:</b>					
Synbiotic			X	X	
Placebo			X	X	
<b>Assessments:</b>					
<b>[List baseline variables]</b>					
Blood pressure, proteinuria, gestational age, weight, height, BMI	X	X			
<b>[List outcome variables]</b>					
Mean systolic blood pressure				X	X
Mean diastolic blood pressure				X	X
Mean duration of pregnancy from diagnosis to delivery				X	
Mean birth weight				X	
<b>[List other data variables]</b>					
Incidence of severe preeclampsia				X	X
Proteinuria				X	X
Anti-hypertensive drug use				X	X
Elective delivery rate				X	
Normal delivery rate				X	
Severity of serious complications				X	X
Average fetal age at termination of pregnancy				X	
Neonatal death					X
Fetal death				X	
Preterm birth				X	
Infant special care of more than 7 days					X
Low birth weight				X	
Average of weight				X	
Average of height				X	
Average of head circumference				X	
Apgar score less than 7 in 5 <sup>th</sup> min				X	
Surfactant use				X	
Intubation and mechanical ventilation				X	
Continuous positive airway pressure				X	
Possible side effects of synbiotic capsules and placebo				X	X

Figure 2. Time schedule of Enrolment, Interventions, and Assessments.

national protocol. Patients will be given a contact number for inquiries or issues. In case of emerging complications that may be related to our intervention, the intervention will be stopped, but monitoring will be continued due to the intention to treat (ITT) approach and the study outcomes will be evaluated. People who receive less than 7 days due to childbirth or unwillingness to take complementary medicine will be reported. From the researcher who will generate the allocation sequence will be inquired about

the type, and complications will be noted. At the end of the study, the quantity of ingested medicines and marked sheets will be checked for calculating the compliance rate of capsules. Paper forms will be used to collect data and the research team will be involved in gathering information. In order to maintain the confidentiality of participants' information, questionnaires with anonymous numbers will be used. Data monitoring committee will monitor this study and if necessary, interim analysis will be performed.

**The Study Outcomes**

*The Primary Outcomes*

The primary outcomes of the study are mean systolic and diastolic blood pressure, duration of pregnancy from diagnosis to delivery, birth weight and, gestational age (Table 1). For achieving these outcomes, the average of all measurements of systolic and diastolic blood pressure every day from diagnosis until delivery will be compared between groups. The other our aim to investigate how our intervention impacts the length of pregnancy and average fetal age at delivery. To account for the confounding effect of gestational age, we will stratify based on when mild preeclampsia was diagnosed (early or late, i.e. before or after 34 weeks gestation). Then, in the analysis of outcome (comparison of duration of pregnancy from diagnosis to delivery, and fetal age at delivery between groups), at the time of diagnosis we will adjust the gestational age. At birth, a lever scale (Seca, Germany) will be used to

measure birth weight with minimal coverage.

*The Secondary Outcomes*

The secondary outcomes related to maternity include proteinuria, incidence of severe preeclampsia, antihypertensive drug usage, elective delivery rate (labor induction or cesarean section), vaginal delivery rate, severity of serious complications (HELLP syndrome, stroke, kidney failure, liver failure, intravascular coagulation, pulmonary edema), maternal blood factors, and serum levels of liver enzymes such as ALT, AST, bilirubin, and LDH. The neonatal secondary outcomes are neonatal death, fetal death, preterm birth, infant intensive care of more than seven days, LBW, the average weight, height, and head circumference, Apgar score less than seven in 5th min, surfactant use, intubation, and mechanical ventilation, continuous positive airway pressure, possible side effects of synbiotic capsules and

**Table 1.** Primary and Secondary Outcomes of the Study

	Objectives	
	Primary Outcomes	Secondary Outcomes
Mother objectives	Mean systolic blood pressure	Proteinuria
	Mean diastolic blood pressure	Incidence of severe preeclampsia
	Mean duration of pregnancy from diagnosis to delivery	Antihypertensive drug use
		Elective delivery rate
		Vaginal delivery rate
		Severity of serious complications
		Side effects of synbiotic capsules
		Serum creatinine
		Platelet count
		ALT
AST		
Bilirubin		
LDH		
Neonatal objective	Mean birth weight	Neonatal death
	Gestational age	Fetal death
		Preterm birth
		Infant intensive care of more than 7 days
		LBW
		Average weight
		Average height
		Average head circumference
		Apgar score less than seven in 5 <sup>th</sup> min
		Surfactant use
Intubation		
Mechanical ventilation		
Continuous positive airway pressure		

placebo (Table 1). These outcomes will be complemented by study measurement tools (described in the previous section) based on clinical and laboratory examinations and information in the patient's medical records. The height of the newborn will be measured without shoes or hats using a stadiometer table while they are in the supine position. The neonatal head circumference will be measured using a tape measure from the occipital prominence to the forehead, without any headwear. Birth weight below 2500 g is considered as LBW. Labor occurring between 20 to 37 weeks of pregnancy will be considered as preterm labor.

### Data Management

To manage the data, unique codes will be given to individuals from 1 to 128 and will be recorded in their questionnaires. The participants data will enter into the SPSS software with the same code. Information will be collected from valid questionnaires and will be entered into SPSS software by two people separately to guarantee the precision and quality of the data. The entry made by the second individual, also known as the second pass entry, plays a pivotal role in verifying and reconciling data by detecting transcription errors and discrepancies arising from illegible data (26). The data of people relating to protocol non-adherence, will be included in the analysis.

According to national protocol for mild preeclampsia, in case of clinical diagnosis, the management routinely includes hospital admission and maternal and fetal close supervision, and decisions are made based on gestational age. In the event of severe preeclampsia, termination of pregnancy will be performed and included in the analysis.

Primary care includes evaluation of signs and symptoms (headache, visual disorder, epigastric pain, and sudden weight gain of about 1.5 kg/wk), measurement of uterine height and gestational age, daily weighing, relative rest, protein-rich diet, high-calorie food, sitting blood pressure monitor every 4 hours (except midnight to 6 am if blood pressure is controlled), using the ISOMED mercury barometer. It will be measured with a  $\pm 3$  mm Hg error.

Urine protein measurement at admission and if the proteinuria is +1 or higher or the protein/creatinine ratio is 0.3 or higher, 24-hour urine collection will be performed.

In the case of 24-hour urine proteinuria, no further tests will be required to repeat the test, and serum creatinine levels will be sufficient to evaluate renal function. CBC measurements, especially platelets, creatinine, liver enzymes, bilirubin, LDH testing will be repeated two to three times weekly, depending on maternal conditions and severity of blood pressure.

Fetal health assessments include daily heart rate hearing, fetal movement control, an initial ultrasound to monitor fetal growth and then every three weeks, fetal health control with biophysical profiles (amniotic fluid index and non-stress test): The interval between tests will depend on the gestational age, the severity of the disease

and intrauterine growth disorder, amniotic fluid index and fetal Doppler changes.

If the duration of pregnancy is under 37 weeks, symptoms should be reviewed regularly and primary care will be provided until delivery.

Pregnancy can be terminated if the gestational age is 37 weeks or more. Magnesium sulfate will be injected in the active phase of the patient's delivery.

In this study, an academic staff as a representative of the ethics committee will monitor research day-to-day and will report the process of intervention to the ethics committee in three stages: The beginning of the intervention, during the intervention, data analysis, and final reporting. She/ he is independent of sponsors and competing interests.

### Statistical Analysis

The results will be entered into SPSS 24 and the normality of the quantitative data will be analyzed through the Kolmogorov-Smirnov test. The study will utilize the general linear model adjusted for baseline and potential confounders to compare mean quantitative variables among groups.

A binary logistic regression model will be utilized for comparison of binary variables between study groups, with adjustment for potential confounding variables. All calculations will be based on ITT and  $\alpha=0.05$  with a 95% confidence interval at all stages. Random assignment of individuals to groups will greatly control the confounding variables. If there be significant disparities between the study groups in the baseline characteristics (demographic and obstetric variables), they will be statistically adjusted. The important baseline characteristics such as the baseline value of outcomes (baseline blood pressure and proteinuria), age, and body mass index, vitamin D3 intake, ... (according to the clinical justification) will be adjusted. For managing missing data, we considered 10% of the sample drop for each group. If the drops are more than 10%, the expectation-maximization will be used for the estimation of missing values.

### Discussion

Preeclampsia is a condition that endangers the life of both the mother and fetus, with severe maternal and perinatal complications. If the treatment is found, it could have a major impact on reducing the complications of this disorder and increasing the health of mothers and babies. Currently, no effective treatment exists except for delivery of the fetus and placenta (27). The suggested therapies that have reached human trials are very few. In many countries, preventive interventions can be quite valuable, even with small or medium benefits.

Preeclampsia is linked to oxidative stress and inflammation. A potential treatment for preeclampsia may involve a drug that reduces soluble fms-like tyrosine kinase 1 (sFlt-1) and soluble endoglin (sEng), as well

as endothelial dysfunction and oxidative stress. In this study, considering the etiology of preeclampsia and the mechanism of the effect of probiotic and synbiotic supplementation, it is suggested that these supplements can be prescribed as a possible treatment for preeclampsia. There are no anticipated problems about probiotics that are detrimental to the participant (22).

We plan to enroll 128 women with preeclampsia at Al-Zahra hospital, located in downtown Tabriz. The reason for choosing this hospital for sampling is that this hospital is a third-level referral center that has a neonatal and maternal special care unit. It is an academic center, with most pregnant mothers suspected of having preeclampsia belonging to various socio-economic levels in the province are referred to this center. The results might potentially be generalizable to developed and developing countries.

Previous studies of esomeprazole for early-onset preeclampsia have suggested that it is a class-C drug during pregnancy is classified by the Food and Drug Administration (FDA). Group-C pertains to pharmaceuticals that have been observed to have unfavorable impact on animal fetal reproduction, lacking sufficient and appropriately controlled studies in humans (27).

Pravastatin has also been suggested for early preeclampsia and at-risk women. However, the drug is assigned to Group-X in pregnancy by the FDA classification system and database for the administration of pregnancy drugs. FDA-class-X drugs are medications which have been tested in animals or humans and have demonstrated a risk of fetal abnormalities and/or harm to humans during pregnancy (28).

Probiotics have great potential as therapeutic approaches in pregnancy and have many beneficial effects in controlling atopic disorders (29). Observational studies indicate a correlation between consuming milk with probiotics and a decreased likelihood of preeclampsia and preterm labor (21).

These dietary supplements can reduce systemic inflammation and oxidative stress (19), and promote digestive health by suppressing pathogenic bacteria. They also modify physiological processes related to hypertension, inflammation, kidney function, and diabetes (20). The most important probiotic success factors in the treatment of inflammation, oxidative stress, and metabolism depend on the type and origin of the process. The probiotic *Lactobacillus* and *Bifidobacterium* species have been extensively researched and used. *Lactobacillus* is especially known as anti-inflammatory, anti-oxidative, anticoagulant, anticancer, and anti-diabetic (30, 31). Previous studies have demonstrated the anti-inflammatory, anti-oxidant, anti-lipidemic, and immune-enhancing properties of *Bifidobacterium* species (32).

Probiotic strains are able to limit a large amount of free reactive radicals in the body and help prevent and control several oxidative stress-related diseases. In general,

these supplements affect inflammatory biomarkers and oxidative stress (19).

The mechanism of the probiotic's anti-inflammatory response is through the modulation of the expression of genes related to gut inflammation and blood pressure, such as the mechanism of anti-inflammatory drugs (29). They also decrease the expression of lipopolysaccharide of gram-negative bacteria, thereby reducing their inflammation, especially the inflammation in human placental trophoblast cells (33).

Another mechanism of the effect of probiotics is their antioxidant activity by inhibiting the oxidation process (34). Probiotics have the ability to generate certain B-group vitamins, which possess antioxidant characteristics (35) and are instrumental in the removal of superoxide and hydroxyl radicals (36). It has also been suggested that probiotic bacteria generate short-chain fatty acid, which blocks the synthesis of serum high-sensitivity C-reactive protein by blocking hepatic enzymatic synthesis (37). Probiotics also regulate the production of fatty acids (38), thereby, the occurrence of inflammatory enzymes is hindered and the antioxidant status is enhanced (39). Some biological peptides produced by probiotics have disinfectant activities and kill free radicals (40).

A systematic review, entitled "Probiotics in Pregnancy and Maternal Results" was conducted in 2013, including randomized controlled trials and prospective cohort studies. This study demonstrated that the utilization of probiotics exhibits a safeguarding impact on the prevalence of preeclampsia. In a cohort study, probiotic-containing dairy products were linked to a lower risk of preeclampsia, especially severe preeclampsia. The results of this review study also reported significant positive effects of the probiotic on lowering maternal blood glucose and decreasing insulin intake as well as incidence of gestational diabetes (29).

A triple-blind controlled clinical trial was conducted to evaluate the effect of probiotic supplementation on blood pressure in pregnant women with gestational diabetes mellitus. The aim of this study was to determine the effect of probiotic supplementation on factors such as systolic blood pressure (SBP) and diastolic blood pressure (DBP). The intervention group did not have increased blood pressure compared to baseline. However, the control group had significantly higher SBP and DBP than at baseline (41). It has been shown that probiotic and synbiotic supplements can significantly reduce hepatic statuses, ALT, AST and improve conditions of liver (42,43), serum creatinine (44), proteinuria (45), bilirubin (46). Prebiotics promote the growth or activity of beneficial bacterial species (probiotics) in the gut and, on the other hand, inhibit the establishment and proliferation of certain pathogenic bacteria such as Salmonella. Moreover, as a source of fermentable substrates that support the proliferation of probiotic microorganisms, particularly lactobacilli and bifidobacteria, dietary



fiber supplementation has numerous advantageous physiological impacts. Additionally, probiotic encapsulation agents are utilized to enhance the stability of these microorganisms in food formulations (23).

As for the safety of probiotic and prebiotic supplements, these supplements are unlikely to enter the body's circulatory system and damage the immune system (47). Studies in pregnancy have so far shown no adverse effects on the mother and fetus other than their positive effects. Because these supplements are used for the treatment after the first trimester, they are unlikely to affect angiogenesis. Their systemic absorption is very rare and no risk has been reported (22).

Considering that this disease is considered as a global-health problem and is related with a very high rate of morbidity and mortality of mothers and neonatal, in many countries, preventive and therapeutic interventions, even with small or moderate benefits, can be considered very valuable.

### Study Limitations

1) In premature infants, the Apgar score is not a very good criterion for diagnosing neonatal asphyxia (27).

2) There are several gynecologists and pregnancy termination decisions. But given that the training centers run the patients according to the protocol of the Ministry of Health and treatment, this limitation will be somewhat manageable.

3) We will compare a synbiotic supplementation with the placebo. No probiotic group is considered.

### Authors' Contribution

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### Conflict of Interests

The authors declare that they have no competing interests.

### Data Availability Statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

### Disclaimer

The funder will provide proper arrangements in place to initiate the study

and will monitor study progress, but will not have a role in the conduct of the study and not contribute to the preparation of this manuscript. The study protocol has undergone peer review of the funding institute.

### Ethical Issues

Written informed consents will be obtained from participants. The Ethics Committee of Tabriz University of Medical Sciences has approved the protocol (code of approved ethics: IR.TBZMED.REC.1398.556). Besides, This protocol has been registered at Iranian Registry of Clinical Trials (identifier: IRCT20110606006709N20; <https://www.irct.ir/trial/39930>).

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### References

1. von Dadelszen P, Firoz T, Donnay F, et al. Preeclampsia in low and middle income countries-health services lessons learned from the PRE-EMPT (PRE-eclampsia-eclampsia monitoring, prevention and treatment) project. *J Obstet Gynaecol Can.* 2012;34(10):917-926. doi:10.1016/s1701-2163(16)35405-6
2. Macedo TCC, Montagna E, Trevisan CM, et al. Prevalence of preeclampsia and eclampsia in adolescent pregnancy: a systematic review and meta-analysis of 291,247 adolescents worldwide since 1969. *Eur J Obstet Gynecol Reprod Biol.* 2020;248:177-186. doi:10.1016/j.ejogrb.2020.03.043
3. Bilano VL, Ota E, Ganchimeg T, Mori R, Souza JP. Risk factors of pre-eclampsia/eclampsia and its adverse outcomes in low- and middle-income countries: a WHO secondary analysis. *PLoS One.* 2014;9(3):e911198. doi:10.1371/journal.pone.0091198
4. Cardoso PM, Surve S. The effect of vitamin E and vitamin C on the prevention of preeclampsia and newborn outcome: a case-control study. *J Obstet Gynaecol India.* 2016;66(Suppl 1):271-278. doi:10.1007/s13224-016-0885-z
5. Atallah A, Lecarpentier E, Goffinet F, Doret-Dion M, Gaucherand P, Tsatsaris V. Aspirin for prevention of preeclampsia. *Drugs.* 2017;77(17):1819-1831. doi:10.1007/s40265-017-0823-0
6. Yuan J, Yu Y, Zhu T, Lin X, Jing X, Zhang J. Oral magnesium supplementation for the prevention of preeclampsia: a meta-analysis or randomized controlled trials. *Biol Trace Elem Res.* 2022;200(8):3572-3581. doi:10.1007/s12011-021-02976-9
7. Bezerra Maia e Holanda Moura S, Marques Lopes L, Murthi P, da Silva Costa F. Prevention of preeclampsia. *J Pregnancy.* 2012;2012:435090. doi:10.1155/2012/435090
8. Berzan E, Doyle R, Brown CM. Treatment of preeclampsia: current approach and future perspectives. *Curr Hypertens Rep.* 2014;16(9):473. doi:10.1007/s11906-014-0473-5
9. Sibai BM. Diagnosis and management of gestational hypertension and preeclampsia. *Obstet Gynecol.* 2003;102(1):181-192. doi:10.1016/s0029-7844(03)00475-7
10. Hubel CA. Oxidative stress in the pathogenesis of preeclampsia. *Proc Soc Exp Biol Med.* 1999;222(3):222-235. doi:10.1046/j.1525-1373.1999.d01-139.x
11. Morken NH, Vogel I, Kallen K, et al. Reference population for international comparisons and time trend surveillance of preterm delivery proportions in three countries. *BMC Womens Health.* 2008;8:16. doi:10.1186/1472-6874-8-16
12. Goswami D, Tannetta DS, Magee LA, et al. Excess syncytiotrophoblast microparticle shedding is a feature of early-onset pre-eclampsia, but not normotensive intrauterine growth restriction. *Placenta.* 2006;27(1):56-61. doi:10.1016/j.placenta.2004.11.007
13. Dusse LM, Rios DR, Pinheiro MB, Cooper AJ, Lwaleed BA. Preeclampsia: relationship between coagulation, fibrinolysis and inflammation. *Clin Chim Acta.* 2011;412(1-2):17-21. doi:10.1016/j.cca.2010.09.030
14. Walsh SW. Plasma from preeclamptic women stimulates transendothelial migration of neutrophils. *Reprod Sci.* 2009;16(3):320-325. doi:10.1177/1933719108327594
15. Baydas G, Karatas F, Gursu MF, et al. Antioxidant vitamin levels in term and preterm infants and their relation to maternal vitamin status. *Arch*

- Med Res. 2002;33(3):276-280. doi:10.1016/s0188-4409(02)00356-9
16. El-Sayed AAF. Preeclampsia: a review of the pathogenesis and possible management strategies based on its pathophysiological derangements. *Taiwan J Obstet Gynecol.* 2017;56(5):593-598. doi:10.1016/j.tjog.2017.08.004
  17. Cani PD, Delzenne NM. Gut microflora as a target for energy and metabolic homeostasis. *Curr Opin Clin Nutr Metab Care.* 2007;10(6):729-734. doi:10.1097/MCO.0b013e3282efdebb
  18. Behnsen J, Deriu E, Sassone-Corsi M, Raffatelli M. Probiotics: properties, examples, and specific applications. *Cold Spring Harb Perspect Med.* 2013;3(3):a010074. doi:10.1101/cshperspect.a010074
  19. Hajifaraji M, Jahanjou F, Abbasalizadeh F, Aghamohammadzadeh N, Mesgari Abbasi M, Dolatkah N. Effect of probiotic supplements in women with gestational diabetes mellitus on inflammation and oxidative stress biomarkers: a randomized clinical trial. *Asia Pac J Clin Nutr.* 2018;27(3):581-591. doi:10.6133/apjcn.082017.03
  20. Lye HS, Kuan CY, Ewe JA, Fung WY, Liong MT. The improvement of hypertension by probiotics: effects on cholesterol, diabetes, renin, and phytoestrogens. *Int J Mol Sci.* 2009;10(9):3755-3775. doi:10.3390/ijms10093755
  21. Brantsaeter AL, Myhre R, Haugen M, et al. Intake of probiotic food and risk of preeclampsia in primiparous women: the Norwegian Mother and Child Cohort Study. *Am J Epidemiol.* 2011;174(7):807-815. doi:10.1093/aje/kwr168
  22. Elias J, Bozzo P, Einarson A. Are probiotics safe for use during pregnancy and lactation? *Can Fam Physician.* 2011;57(3):299-301.
  23. Fuentes-Zaragoza E, Sánchez-Zapata E, Sendra E, et al. Resistant starch as prebiotic: a review. *Stärke.* 2011;63(7):406-415. doi:10.1002/star.201000099
  24. Chappell LC, Seed PT, Briley AL, et al. Effect of antioxidants on the occurrence of pre-eclampsia in women at increased risk: a randomised trial. *Lancet.* 1999;354(9181):810-816. doi:10.1016/s0140-6736(99)80010-5
  25. Cluver CA, Hannan NJ, van Papendorp E, et al. Esomeprazole to treat women with preterm preeclampsia: a randomized placebo controlled trial. *Am J Obstet Gynecol.* 2018;219(4):388.e1-388.e17. doi:10.1016/j.ajog.2018.07.019
  26. Krishnankutty B, Bellary S, Kumar NB, Moodahadu LS. Data management in clinical research: an overview. *Indian J Pharmacol.* 2012;44(2):168-172. doi:10.4103/0253-7613.93842
  27. Cluver CA, Walker SP, Mol BW, et al. Double blind, randomised, placebo-controlled trial to evaluate the efficacy of esomeprazole to treat early onset pre-eclampsia (PIE Trial): a study protocol. *BMJ Open.* 2015;5(10):e008211. doi:10.1136/bmjopen-2015-008211
  28. Costantine MM, Tamayo E, Lu F, et al. Using pravastatin to improve the vascular reactivity in a mouse model of soluble FMS-like tyrosine kinase-1-induced preeclampsia. *Obstet Gynecol.* 2010;116(1):114-120. doi:10.1097/AOG.0b013e3181e10ebd
  29. Lindsay KL, Walsh CA, Brennan L, McAuliffe FM. Probiotics in pregnancy and maternal outcomes: a systematic review. *J Matern Fetal Neonatal Med.* 2013;26(8):772-778. doi:10.3109/14767058.2012.755166
  30. Amdekar S, Singh V, Kumar A, Sharma P, Singh R. Lactobacillus casei and Lactobacillus acidophilus regulate inflammatory pathway and improve antioxidant status in collagen-induced arthritic rats. *J Interferon Cytokine Res.* 2013;33(1):1-8. doi:10.1089/jir.2012.0034
  31. Patel B, Kumar P, Banerjee R, et al. Lactobacillus acidophilus attenuates *Aeromonas hydrophila* induced cytotoxicity in catla thymus macrophages by modulating oxidative stress and inflammation. *Mol Immunol.* 2016;75:69-83. doi:10.1016/j.molimm.2016.05.012
  32. Meng H, Ba Z, Lee Y, et al. Consumption of Bifidobacterium animalis subsp. lactis BB-12 in yogurt reduced expression of TLR-2 on peripheral blood-derived monocytes and pro-inflammatory cytokine secretion in young adults. *Eur J Nutr.* 2017;56(2):649-661. doi:10.1007/s00394-015-1109-5
  33. Yeganegi M, Watson CS, Martins A, et al. Effect of Lactobacillus rhamnosus GR-1 supernatant and fetal sex on lipopolysaccharide-induced cytokine and prostaglandin-regulating enzymes in human placental trophoblast cells: implications for treatment of bacterial vaginosis and prevention of preterm labor. *Am J Obstet Gynecol.* 2009;200(5):532.e1-532.e8. doi:10.1016/j.ajog.2008.12.032
  34. Mayo B, van Sinderen D. Bifidobacteria: Genomics and Molecular Aspects. Horizon Scientific Press; 2010.
  35. Li P, Zhou Q, Gu Q. Complete genome sequence of Lactobacillus plantarum LZ227, a potential probiotic strain producing B-group vitamins. *J Biotechnol.* 2016;234:66-70. doi:10.1016/j.jbiotec.2016.07.020
  36. Kullisaar T, Zilmer M, Mikelsaar M, et al. Two antioxidative lactobacilli strains as promising probiotics. *Int J Food Microbiol.* 2002;72(3):215-224. doi:10.1016/s0168-1605(01)00674-2
  37. Asemi Z, Jazayeri S, Najafi M, et al. Effects of daily consumption of probiotic yoghurt on inflammatory factors in pregnant women: a randomized controlled trial. *Pak J Biol Sci.* 2011;14(8):476-482. doi:10.3923/pjbs.2011.476.482
  38. Gargari G, Taverniti V, Balzaretto S, et al. Consumption of a Bifidobacterium bifidum strain for 4 weeks modulates dominant intestinal bacterial taxa and fecal butyrate in healthy adults. *Appl Environ Microbiol.* 2016;82(19):5850-5859. doi:10.1128/aem.01753-16
  39. Yuan X, Wang L, Bhat OM, Lohner H, Li PL. Differential effects of short chain fatty acids on endothelial Nlrp3 inflammasome activation and neointima formation: antioxidant action of butyrate. *Redox Biol.* 2018;16:21-31. doi:10.1016/j.redox.2018.02.007
  40. Saiga A, Tanabe S, Nishimura T. Antioxidant activity of peptides obtained from porcine myofibrillar proteins by protease treatment. *J Agric Food Chem.* 2003;51(12):3661-3667. doi:10.1021/jf021156g
  41. Hajifaraji M, Jahanjou F, Abbasalizadeh F, Aghamohammadzadeh N, Mesgari Abbasi M, Dolatkah N. Effect of probiotic supplementation on blood pressure of females with gestational diabetes mellitus: a randomized double blind controlled clinical trial. *Iran Red Crescent Med J.* 2017;19(6):e55662. doi:10.5812/ircmj.55662
  42. Dugoua JJ, Machado M, Zhu X, Chen X, Koren G, Einarson TR. Probiotic safety in pregnancy: a systematic review and meta-analysis of randomized controlled trials of Lactobacillus, Bifidobacterium, and Saccharomyces spp. *J Obstet Gynaecol Can.* 2009;31(6):542-552. doi:10.1016/s1701-2163(16)34218-9
  43. Khalesi S, Johnson DW, Campbell K, et al. Effect of probiotics and synbiotics consumption on serum concentrations of liver function test enzymes: a systematic review and meta-analysis. *Eur J Nutr.* 2018;57(6):2037-2053. doi:10.1007/s00394-017-1568-y
  44. Dai Y, Quan J, Xiong L, Luo Y, Yi B. Probiotics improve renal function, glucose, lipids, inflammation and oxidative stress in diabetic kidney disease: a systematic review and meta-analysis. *Ren Fail.* 2022;44(1):862-880. doi:10.1080/0886022x.2022.2079522
  45. Yacoub R, Kaji D, Patel SN, et al. Association between probiotic and yogurt consumption and kidney disease: insights from NHANES. *Nutr J.* 2016;15:10. doi:10.1186/s12937-016-0127-3
  46. Zahed Pasha Y, Ahmadpour-Kacho M, Ahmadi Jazi A, Gholinia H. Effect of probiotics on serum bilirubin level in term neonates with jaundice; a randomized clinical trial. *Int J Pediatr.* 2017;5(10):5925-5930. doi:10.22038/ijp. 2017.24996.2117
  47. Dugoua JJ, Machado M, Zhu X, Chen X, Koren G, Einarson TR. Probiotic safety in pregnancy: a systematic review and meta-analysis of randomized controlled trials of Lactobacillus, Bifidobacterium, and Saccharomyces spp. *J Obstet Gynaecol Can.* 2009;31(6):542-552. doi:10.1016/s1701-2163(16)34218-9